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Cryptic diversity across the Trans-Mexican Volcanic Belt of Mexico in the montane bunchgrass lizard *Sceloporus subniger* (Squamata: Phrynosomatidae)

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Abstract

Sceloporus subniger Poglaygen & Smith is a montane bunchgrass lizard distributed across pine-oak forests of central Mexico. Prompted by the discovery of a new population of this lizard in far western Mexico, and by recent studies suggesting *S. subniger* may be a composite of several distinct species, we examined in more detail the genetic structure of *S. subniger*. We generated a mitochondrial DNA (mtDNA) dataset from 81 specimens and an ultraconserved elements (UCE) dataset representing thousands of genomic regions from 12 specimens to specifically evaluate the genetic distinctiveness of populations from western Michoacán and adjacent Jalisco along with the newly discovered population in the Sierra de Mascota in western Jalisco. We also recorded morphological data from 47 museum specimens to compare to our genetic data. Results from our analyses of the genetic data, augmented by specimen measurements and scale counts, support the notion that *S. subniger* is indeed a composite of distinct species. Montane bunchgrass lizards from western Michoacán and adjacent Jalisco, and from the Sierra de Mascota in western Jalisco, each represent distinct new species, which we describe and name here.

Keywords: mitochondrial DNA, new species, phylogenomics, *Sceloporus scalaris* group, ultraconserved elements

Resumen

Sceloporus subniger Poglaygen & Smith es una lagartija llanera de montaña distribuida en los bosques de pino-encino del centro de México. Impulsados por el descubrimiento de una nueva población de la especie en el occidente de México, y por recientes estudios que sugieren que *S. subniger* posiblemente se trate de varias especies distintas, examinamos a detalle la estructura genética de *S. subniger*. Se generó un set de datos de ADN mitocondrial (mtDNA) de 81 ejemplares y un set de datos de elementos ultra-conservados (UCE en inglés) conteniendo miles de regiones genómicas de 12 ejemplares para evaluar específicamente la distintividad genética de las poblaciones del occidente de Michoacán y la región adyacente de Jalisco junto con la población descubierta recientemente en la Sierra de Mascota en el occidente de Jalisco. De igual manera, se analizó información morfológica de 47 especímenes de museo para compararla con nuestra información genética. Los resultados de nuestro análisis de la información genética, además de las mediciones de especímenes y recuentos de escamas, apoyan la idea de que *S. subniger* está compuesta por especies distintas. Las lagartijas llaneras de montaña del oeste de Michoacán y la región adyacente de Jalisco y las provenientes de la Sierra de Mascota en el oeste de Jalisco representan cada una una especie distinta, que describimos y nombramos aquí.

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Introduction

The Mexican highlands are a global biodiversity hotspot with a high level of endemism (Myers *et al*. 2000; Mittermeier *et al*. 2005; Critical Ecosystem Partnership Fund 2016). The Trans-Mexican Volcanic Belt (TVB) is a relatively young volcanic mountain range that stretches across Mexico from the Gulf of Mexico to the Pacific Ocean. Recent studies, aided by molecular data, have described a number of new terrestrial vertebrate species distributed across the TVB (e.g., amphibians: Campbell *et al*. 2018; Grünwald *et al*. 2018; reptiles: Alvarado-Díaz & Campbell 2004; Bryson *et al*. 2014; small mammals: Bradley *et al*. 2017). *Sceloporus subniger* Poglaygen & Smith is a montane bunchgrass lizard in the *Sceloporus scalaris* group that occupies pine-oak forests of the TVB and adjacent highlands. Recent studies have suggested that *S. subniger* may be a composite of several distinct species (Bryson *et al*. 2012; Grummer *et al*. 2014). Species delimitation modeling using 4,365 base pairs of nuclear DNA provided strong evidence that, at a minimum, populations of *S. subniger* distributed across the TVB of western Michoacán and adjacent Jalisco are genetically distinct from all other sampled populations (Grummer *et al*. 2014).

In 2011, we discovered a new population of bunchgrass lizard similar in appearance to *S. subniger* in the Sierra de Mascota in far western Jalisco, over 150 air-km from the closest known localities of *S. subniger* in southeastern Jalisco (Bryson *et al*. 2012). This discovery encouraged us to examine in more detail the genetic structure of *S. subniger* from the central and western regions of the TVB. In particular, we were interested in answering two questions: (1) Should populations from the TVB of western Michoacán and adjacent Jalisco (hereafter referred to as *S. subniger* "West") be recognized as a distinct species, and (2) How do specimens from the Sierra de Mascota relate to other *S. subniger*, and could the Sierra de Mascota population represent a distinct species? We generated two different types of genetic data to address these questions. We sequenced mitochondrial DNA from a large number of individuals collected along the central and western regions of the TVB to assess maternal genetic structure and the degree of haplotype sharing among *S. subniger* "West", *S. subniger*, and specimens from the Sierra de Mascota. From a smaller number of individuals representing the four mitochondrial groups identified in Bryson *et al*. (2012), we mined single nucleotide polymorphisms (SNPs) from thousands of nuclear genomic regions using sequence capture of ultraconserved elements (UCEs) to assess genetic clustering and genomic admixture and to test different species delimitation scenarios. We then measured and compared morphological characters historically used for distinguishing species in the *S. scalaris* group from specimens collected from the central and western regions of the TVB.

Methods

Genetic data. We generated mitochondrial DNA sequence data from 81 *S. subniger* collected from 15 localities centered along the central and western regions of the TVB (Fig. 1, Table 1). This included 42 samples from specimens of *S. subniger* "West" and two specimens from the Sierra de Mascota. We included *S. chaneyi* Liner & Dixon and *S. undulatus* (Bosc & Daudin) as outgroups (Leaché *et al*. 2016). We extracted genomic DNA from liver or tail tissues using Qiagen DNeasy Blood & Tissue Kits (Qiagen Inc.) and amplified an 867 base-pair fragment of the mitochondrial gene NADH dehydrogenase subunit 4 (ND4) and flanking tRNAs using standard protocols (Bryson *et al*. 2012). Polymerase chain reaction products were sent to the High-Throughput Genomics Unit (HGTU, University of Washington) for sequencing. We edited and manually aligned forward and reverse sequences for each individual using Sequencher v.5.0 (Gene Codes Corporation, Ann Arbor, MI). Sequence data were deposited in Dryad (doi:10.5061/dryad.c59zw3r4n).

We generated genomic data from 12 samples representing the four mitochondrial groups of *S. subniger* in Bryson *et al*. (2012) (Fig. 1, Table 2). Eight of these samples were also represented in the mtDNA dataset. We were unable to include samples from the Sierra de Mascota in western Jalisco, which were not available at the time of UCE sequencing. We included two samples of the closely related species *S. bicanthalis* Smith (Grummer *et al*. 2014; Leaché *et al*. 2016) for clustering and species delimitation analyses. We extracted genomic DNA from liver or tail tissues using Qiagen DNeasy Blood & Tissue Kits, quantified extractions using a Qubit 2.0 fluorometer (Life Technologies, Inc.), and sent extractions to RAPiD Genomics (Gainesville, FL, USA) for UCE sequence capture and sequencing. Each pool was enriched using a set of 5,472 custom-designed probes (MYbaits, MYcroarray, Inc.) targeting 5,060 UCE loci (Faircloth *et al*. 2012) following an open-source protocol (see www.ultraconserved.org for the full protocol). Pooled libraries were sent to the University of Florida ICBR Facility for 100 bp paired-end sequencing on an Illumina HiSeq 2500.

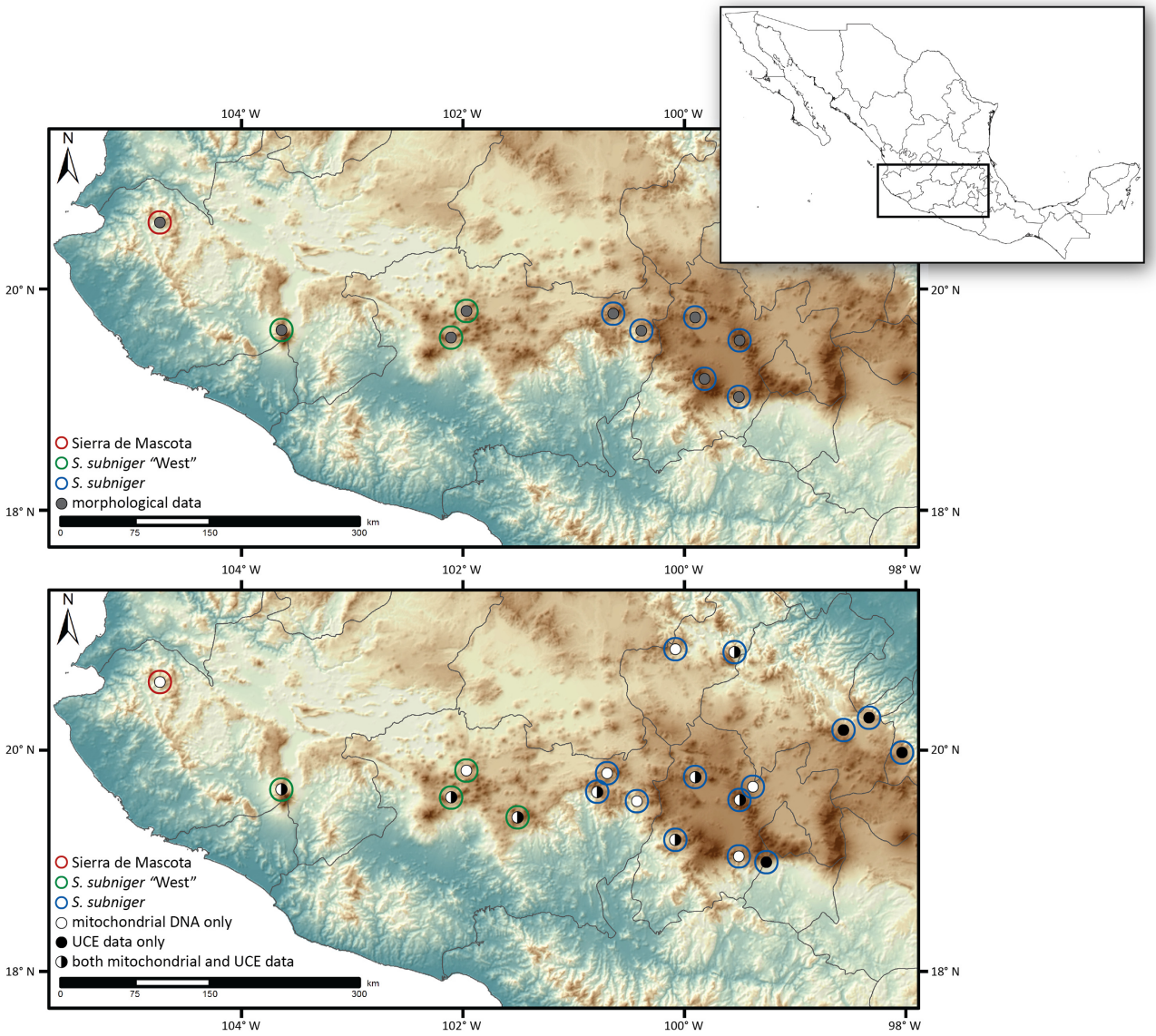

FIGURE 1. Localities of the montane bunchgrass lizard *Sceloporus subniger* sampled for this study. *Sceloporus subniger* "West" refers to the western clade of *S. subniger* identified in previous studies (Bryson *et al*. 2012; Grummer & Bryson 2014; Grummer *et al*. 2014); the Sierra de Mascota represents a newly discovered locality in western Jalisco.

After sequencing, we followed the standard PHYLUCE v.1.5.0 pipeline for processing target-enriched UCE data (Faircloth 2016). We trimmed reads of adapter contamination and low-quality bases with Illumiprocessor (Faircloth 2013) and Trimmomatic (Bolger *et al*. 2014). We then assembled cleaned reads into contigs using Trinity (Grabherr *et al*. 2011), and aligned assembled contigs to the original UCE probe sequences. We then called SNPs from UCE loci for downstream analyses of admixture and species tree estimation. As a reference sequence, we used a closely related outgroup sample (MX186 *S. bicanthalis*) which contained the second highest number of UCE contigs. We used BWA (Li & Durbin 2009) to map individual sample reads to the reference, SAMtools (Li *et al*. 2009) to sort the reads, mark duplicates, and index samples, and Picard (available at http://broadinstitute.github.io/picard) to identify and remove PCR duplicates. We then used Genome Analysis Toolkit (GATK) v.3.2 Best Practices Workflow to realign the mapped reads to minimize mismatched bases due to indels. We followed GATK's best practices for base recalibration for non-model organisms, which involved executing four rounds of base recalibration on the original data to filter out systematic error using the custom script genotyperecal.sh, and calling genotypes based on the last recalibrated BAM file. We used vcf-tools (Danecek *et al*. 2011) to select one SNP per UCE and produce two data sets: one allowing 25% missing data for clustering and admixture analyses, and one with no missing data for species delimitation analyses. Sequence data were deposited in Dryad (doi:10.5061/dryad.c59zw3r4n).

TABLE 1. Samples of the montane bunchgrass lizard *Sceloporus subniger* sequenced for the mitochondrial gene ND4.

Species	Sample Number	Locality
S. subniger	MX269_Aporo	Michoacán: SE Aporo
S. subniger	MX272_MilCumbres	Michoacán: Mil Cumbres
S. subniger	MX278_Toliman	Querétaro: El Derramadero, Mpo. Tolimán
S. subniger	MX280_Vcarbon	Estado de México: Villa del Carbón
S. subniger	MX442_ValleBravo	Estado de México: Valle de Bravo
S. subniger	MX268_ValleBravo	Estado de México: Valle de Bravo
S. subniger	MXH60_ValleBravo	Estado de México: Valle de Bravo
S. subniger	MXH61_ValleBravo	Estado de México: Valle de Bravo
S. subniger	MXH62_ValleBravo	Estado de México: Valle de Bravo
S. subniger	MXH63_ValleBravo	Estado de México: Valle de Bravo
S. subniger	MXH64_ValleBravo	Estado de México: Valle de Bravo
S. subniger	MXH65_ValleBravo	Estado de México: Valle de Bravo
S. subniger	MX270_Azufres	Michoacán: Los Azufres
S. subniger	MXH66_Azufres	Michoacán: Los Azufres
S. subniger	MXH67_Azufres	Michoacán: Los Azufres
S. subniger	MX271_Azufres	Michoacán: Parque Laguna Larga
S. subniger	MXH68_Azufres	Michoacán: Parque Laguna Larga
S. subniger	MX441_Joquicingo	Estado de México: Joquicingo
S. subniger	MXH69_Joquicingo	Estado de México: Joquicingo
S. subniger	MXH70_Joquicingo	Estado de México: Joquicingo
S. subniger	MXH71_Joquicingo	Estado de México: Joquicingo
S. subniger	MXH72_Joquicingo	Estado de México: Joquicingo
S. subniger	MXH73_Joquicingo	Estado de México: Joquicingo
S. subniger	MXH74_Joquicingo	Estado de México: Joquicingo
S. subniger	MXH75_Joquicingo	Estado de México: Joquicingo
S. subniger	MX16_LosTachos	Estado de México: Los Tachos
S. subniger	MXH76_LosTachos	Estado de México: Los Tachos
S. subniger	MXH77_LosTachos	Estado de México: Los Tachos
S. subniger	MXH78_LosTachos	Estado de México: Los Tachos
S. subniger	MXH79_LosTachos	Estado de México: Los Tachos
S. subniger	MXH80_LosTachos	Estado de México: Los Tachos
S. subniger	MX3_Atlacomulco	Estado de México: 3.2 mi SW Atlacomulco
S. subniger	MXH81_Atlacomulco	Estado de México: 3.2 mi SW Atlacomulco
S. subniger	MXH82_Atlacomulco	Estado de México: 3.2 mi SW Atlacomulco
S. subniger	MXH84_Atlacomulco	Estado de México: 3.2 mi SW Atlacomulco
S. subniger	MXH85_Atlacomulco	Estado de México: 3.2 mi SW Atlacomulco

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TABLE 1. (continued)

Species	Sample Number	Locality
S. subniger	MXH86_Atlacomulco	Estado de México: 3.2 mi SW Atlacomulco
S. subniger "West"	MX19_NColima	Jalisco: Nevado de Colima, 13.5 mi W Cd. Guzmán
S. subniger "West"	MXH87_NColima	Jalisco: Nevado de Colima, 13.5 mi W Cd. Guzmán
S. subniger "West"	MXH88_NColima	Jalisco: Nevado de Colima, 13.5 mi W Cd. Guzmán
S. subniger "West"	MXH89_NColima	Jalisco: Nevado de Colima, 13.5 mi W Cd. Guzmán
S. subniger "West"	MXH90_NColima	Jalisco: Nevado de Colima, 13.5 mi W Cd. Guzmán
S. subniger "West"	MXH91_NColima	Jalisco: Nevado de Colima, 13.5 mi W Cd. Guzmán
S. subniger "West"	MXH92_NColima	Jalisco: Nevado de Colima, 13.5 mi W Cd. Guzmán
S. subniger "West"	MXH93_NColima	Jalisco: Nevado de Colima, 13.5 mi W Cd. Guzmán
S. subniger "West"	MXH94_NColima	Jalisco: Nevado de Colima, 13.5 mi W Cd. Guzmán
S. subniger "West"	MXH95_NColima	Jalisco: Nevado de Colima, 13.5 mi W Cd. Guzmán
S. subniger "West"	MX13_Zacapu	Michoacán: 11.7 mi W Zacapu on rd to Zamora
S. subniger "West"	MXH98_Zacapu	Michoacán: 11.7 mi W Zacapu on rd to Zamora
S. subniger "West"	MXH99_Zacapu	Michoacán: 11.7 mi W Zacapu on rd to Zamora
S. subniger "West"	MXH100_Zacapu	Michoacán: 11.7 mi W Zacapu on rd to Zamora
S. subniger "West"	MXH101_Zacapu	Michoacán: 11.7 mi W Zacapu on rd to Zamora
S. subniger "West"	MXH102_Zacapu	Michoacán: 11.7 mi W Zacapu on rd to Zamora
S. subniger "West"	MXH103_Zacapu	Michoacán: 11.7 mi W Zacapu on rd to Zamora
S. subniger "West"	MXH104_Zacapu	Michoacán: 11.7 mi W Zacapu on rd to Zamora
S. subniger "West"	MX274_Uruapan	Michoacán: 22 km N Uruapan on Hwy 37
S. subniger "West"	MXH105_Uruapan	Michoacán: 22 km N Uruapan on Hwy 37
S. subniger "West"	MXH106_Uruapan	Michoacán: 22 km N Uruapan on Hwy 37
S. subniger "West"	MXH107_Uruapan	Michoacán: 22 km N Uruapan on Hwy 37
S. subniger "West"	MXH108_Uruapan	Michoacán: 22 km N Uruapan on Hwy 37
S. subniger "West"	MX184_SanJoaquin	Querétaro: Nuevo San Joaquín
S. subniger "West"	MXH109_SanJoaquin	Querétaro: Nuevo San Joaquín
S. subniger "West"	MXH110_SanJoaquin	Querétaro: Nuevo San Joaquín
S. subniger "West"	MXH111_SanJoaquin	Querétaro: Nuevo San Joaquín
S. subniger "West"	MXH112_SanJoaquin	Querétaro: Nuevo San Joaquín
S. subniger "West"	MXH113_SanJoaquin	Querétaro: Nuevo San Joaquín
S. subniger "West"	MXH114_SanJoaquin	Querétaro: Nuevo San Joaquín
S. subniger "West"	MXH115_SanJoaquin	Querétaro: Nuevo San Joaquín
S. subniger "West"	MXH116_SanJoaquin	Querétaro: Nuevo San Joaquín
S. subniger "West"	MXH117_SanJoaquin	Querétaro: Nuevo San Joaquín
S. subniger "West"	MX275_SanGregorio	Michoacán: San Gregorio
S. subniger "West"	MXH118_SanGregorio	Michoacán: San Gregorio

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TABLE 1. (continued)

Species	Sample Number	Locality
S. subniger "West"	MXH119_SanGregorio	Michoacán: San Gregorio
S. subniger "West"	MXH120_SanGregorio	Michoacán: San Gregorio
S. subniger "West"	MXH121_SanGregorio	Michoacán: San Gregorio
S. subniger "West"	MXH122_SanGregorio	Michoacán: San Gregorio
S. subniger "West"	MXH123_SanGregorio	Michoacán: San Gregorio
S. subniger "West"	MXH124_SanGregorio	Michoacán: San Gregorio
S. subniger "West"	MXH125_SanGregorio	Michoacán: San Gregorio
S. subniger Sierra de Mascota	MXH96_Mascota	Jalisco: Sierra de Mascota
S. subniger Sierra de Mascota	MXH97_Mascota	Jalisco: Sierra de Mascota

TABLE 2. Samples of the montane bunchgrass lizard *Sceloporus subniger* sequenced for ultraconserved elements (UCEs). Samples marked with an asterisk were also sequenced for the mitochondrial gene ND4. Mitochondrial group designations follow Bryson *et al*. (2012).

Species	Sample Number	Mitochondrial Group	Locality
S. bicanthalis	MX186o	outgroup	Oaxaca: Corral del Piedra
S. bicanthalis	MX286o	outgroup	Puebla: Volcán Iztaccíhuatl
S. subniger	MX16c*	Central	Estado de México: Los Tachos
S. aeneus	MX183c	Central	Morelos: Zempoala
S. subniger	MX184c*	Central	Querétaro: Nuevo San Joaquín
S. subniger	MX272c*	Central	Michoacán: Mil Cumbres
S. subniger	MX3c*	Central	Estado de México: 3.2 mi SW Atlacomulco
S. subniger	MX276e	East	Hidalgo: Palo Gacho
S. subniger	MX277e	East	Hidalgo: Autodromo del Angel
S. subniger	MX423e	East	Puebla: Carretera "Pue 108" Jicolapa y Xochicuautla
S. subniger	MX442s*	South	Estado de México: Valle de Bravo
S. subniger "West"	MX19w*	West	Jalisco: Nevado de Colima, 13.5 mi W Cd. Guzmán
S. subniger "West"	MX274w*	West	Michoacán: 22 km N Uruapan on Hwy 37
S. subniger "West"	MX275w*	West	Michoacán: San Gregorio

Mitochondrial DNA analysis. We generated a mitochondrial phylogeny to assess maternal genetic structure using BEAST v1.8.4 (Drummond *et al*. 2012). Although mtDNA genes only track the history of female genealogy and dispersal, this marker has been useful for revealing the presence of hybridization and gene flow in *Sceloporus* (e.g., Leaché & Cole 2007; Leaché & Mulcahy 2007; Grummer *et al*. 2014; Grummer *et al*. 2015). Our sampling of *S. subniger* was centered on populations near the clade boundaries of *S. subniger* "West" and *S. subniger* along the TVB of Michoacán and Estado de México (Bryson *et al*. 2012). Samples from the newly discovered population in the Sierra de Mascota in western Jalisco were also included. We used W-IQ-TREE v.1.6.1 (Trifinopoulos *et al*. 2016) to select the best-fit model of sequence evolution, based on Bayesian Information Criteria (BIC), for the ND4 gene fragment (HKY+G) and adjacent tRNA region (HKY+I). We used a lognormal relaxed clock model and a constant-size coalescent tree prior, and ran analyses for 40 million generations, retaining trees and parameters every 1,000 steps. Results were displayed in Tracer v.1.6 (Rambaut & Drummond 2007) to assess convergence and ensure

effective sample sizes were above 200 for all estimated parameters. We discarded the first 25% of trees as burn-in and created the maximum-clade credibility tree using TreeAnnotator v.1.8.4 (Drummond *et al*. 2012).

Phylogenomic DNA analyses. We used STRUCTURE v.2.3.4 (Pritchard *et al*. 2000; Falush *et al*. 2003; Falush *et al*. 2007) to evaluate genetic clustering and evidence of genomic admixture among the four mitochondrial groups of *S. subniger* using SNPs from our UCE data. These groups included *S. subniger* "West", *S. subniger* Central (southern Central Mexican Plateau and adjacent slopes of the TVB), *S. subniger* South (southern slopes of the TVB in Estado de México), and *S. subniger* East (southeastern regions of the Sierra Madre Oriental). No nuclear data were available for samples from the Sierra de Mascota in western Jalisco. We used an admixture model with correlated allele frequencies and ran analyses of K=2–5 for 1 million generations each after a 10% burn-in. We ran each analysis four times, and considered the iteration with the highest likelihood to represent the optimal run for that K. We viewed results in STRUCTURE HARVESTER v.0.6.94 (Earl & Vonholdt 2012), and noted the optimal value of K suggested by the Evanno method (Evanno *et al*. 2005; but see Janes *et al*. 2017). Since the Evanno method does not allow the assessment of $K=1$ as a potential solution, *S. bicanthalis* was included in all analyses to evaluate $K=2$ scenarios that cluster samples of *S. subniger* together.

Using SNPs from our UCE data, we also tested two species delimitation scenarios using BFD* (Grummer *et al*. 2014, Leaché *et al*. 2014), implemented with the SNAPP v.1.3.0 (Bryant *et al*. 2012) plugin for BEAST v.2.4.3 (Bouckaert *et al*. 2014). We tested two competing models. The first contained two species: the outgroup (two samples of *S. bicanthalis*), and all *S. subniger* lumped together. The second model consisted of three species: the outgroup, *S. subniger* "West", and the remaining *S. subniger*. For each model, we set the unsampled mutation rates u and v to 1, alpha to 1, beta to 250, lambda to 25, sampled a coalescence rate initially set to 10, and used default settings for all other parameters. We conducted path sampling for a total of 24 steps, running each for 600,000 MCMC generations and sampling every 1,000 steps to estimate marginal likelihoods for the two competing models. We compared the resulting marginal likelihood values using Bayes factors (Bf; Kass & Raftery 1995), and considered the *S. subniger* "West" lineage distinct if the three-species model was strongly supported over the other model ($2ln\text{Bf} > 10$).

Morphological data. We examined a total of 47 adult *S. subniger* from the TVB of Jalisco, Michoacán, and Estado de México (Appendix 1). Morphological comparisons were made among 28 specimens from eastern Michoacán and Estado de México, 14 specimens from western Michoacán and eastern Jalisco (*S. subniger* "West"), and five specimens from the Sierra de Mascota in western Jalisco (Fig. 1). Only individuals over 40 mm snout–vent length, the approximate size at sexual maturity (Thomas & Dixon 1976), were studied. We examined 4 mensural and 14 meristic characters historically used for distinguishing species in the *S. scalaris* group (Thomas & Dixon 1976; Smith *et al*. 1997), and compared the mean values or mode and range of variation among these characters. We measured snout–vent length (from tip of snout to posterior margin of cloacal opening), head length (from tip of snout to posterior edge of interparietal), head width, and tibia length (measured on left leg from top of tibia to where tibia meets tarsus). Snout-vent length and tibia length were measured using a ruler to the nearest mm; head length and width were measured using digital calipers to the nearest 0.01 mm. From these measurements we calculated head width/length and tibia length/head length ratios. We counted the number of dorsal scales (from posterior margin of interparietal to level of posterior insertion of thighs), scales around mid-body (halfway between axilla and groin regions), ventral scales (from incomplete gular fold to anterior margin of cloaca), canthals, loreals, supralabials, infralabials, frontoparietals, parietals, scales bordering interparietal, scales between second pair of postmentals, scales between third pair of postmentals, femoral pores, and subdigital lamellae on fourth toe. Meristic asymmetry was noted as right/left. Terminology for scalation follows Smith (1939). Museum acronyms follow Sabaj Pérez (2013).

Results and discussion

Mitochondrial DNA analysis. Bayesian phylogenetic analysis of mitochondrial DNA placed *S. subniger* from the central and western TVB into three geographical clades (Fig. 2). Samples from the Sierra de Mascota formed a distinct clade, as did samples of *S. subniger* "West". The remaining samples from the central TVB formed a large clade ("*S. subniger* clade"). Additional geographic structure was present within the *S. subniger* clade, with samples from the southern slopes of the TVB in Estado de México (Valle de Bravo and Joquicingo*; S. subniger* South) grouping together, forming the sister taxon to the clade of the remaining samples (*S. subniger* Central + *S. subniger* East). No haplotypes were shared among the three geographical clades.

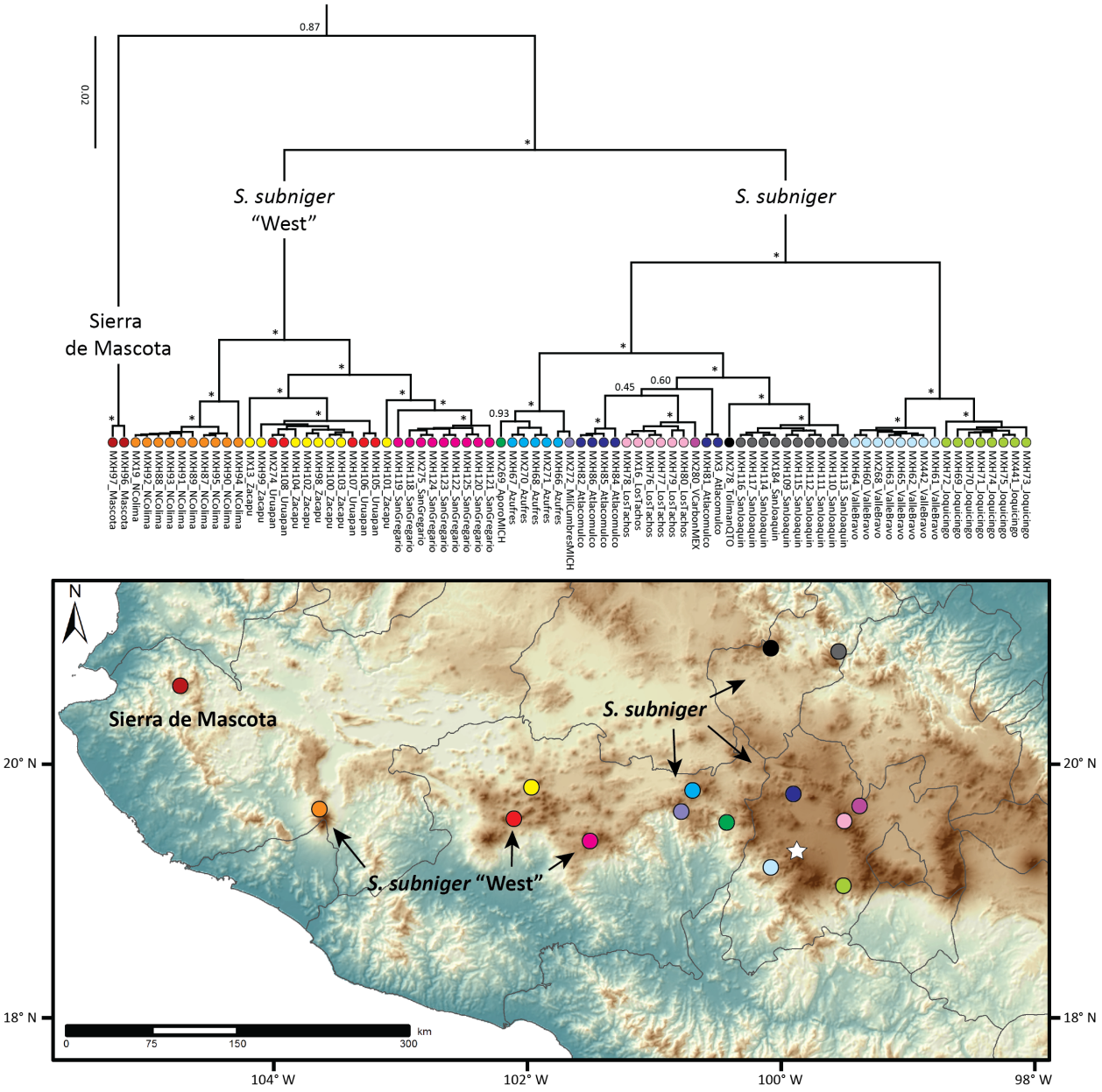

FIGURE 2. Mitochondrial phylogeny of the montane bunchgrass lizard *Sceloporus subniger*. Nodes that received ≥0.95 posterior probability support are indicated with asterisks; posterior probability values for all other nodes are provided. Star denotes the type locality of *S. subniger*. Locality data for each sample can be found in Table 1.

Phylogenomic DNA analyses. After removing non-biallelic loci, selecting one SNP for every UCE locus, and filtering loci for missing data, the STRUCTURE dataset contained 3,324 SNPs and the SNAPP dataset contained 3,223 SNPs. STRUCTURE analyses grouped samples of *S. subniger* "West" into a distinct cluster for all *K* values (Fig. 3), with *K*=4 selected as the optimal *K* value based on the Evanno method. No admixture was inferred between *S. subniger* "West" and other *S. subniger* at any *K* value. Species delimitation using BFD* provided overwhelming support (2*ln*Bf = 5,764.6) for a three-species model that split *S. subniger* "West" from other *S. subniger* over a two-species model that grouped all *S. subniger* into a single species.

Morphological data. Morphological comparisons among *S. subniger* from the TVB of Jalisco, Michoacán, and Estado de México revealed overlapping scale counts and measurements, consistent with previous studies showing little morphological differentation among species in the group (Thomas & Dixon 1976; Grummer & Bryson 2014). However, some combinations of characters were found that weakly distinguish *S. subniger* "West" and specimens from the Sierra de Mascota from each other and from other *S. subniger* (Tables 3–4). *Sceloporus subniger* from eastern Michoacán and Estado de México were larger lizards, with several individuals measuring over 60 mm SVL

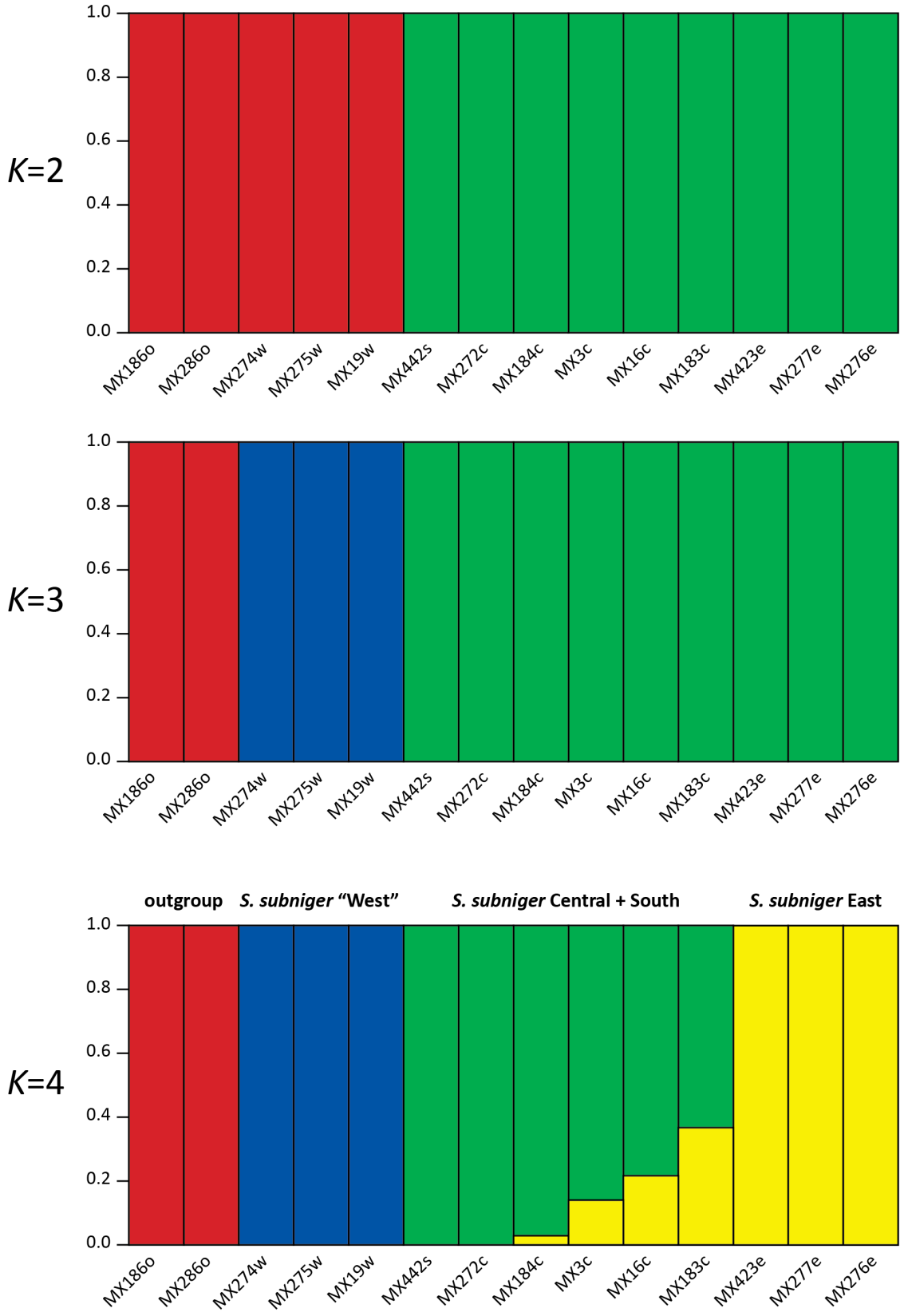

FIGURE 3. Results of STRUCTURE analyses of 3,324 SNPs showing inferred genetic clusters of the montane bunchgrass lizard *Sceloporus subniger*. The optimal number of genetic clusters was *K*=4 based on the Evanno method. Samples represent the four mitochondrial groups of *S. subniger* in Bryson *et al*. (2012), here referred to as *S. subniger* "West", *S. subniger* Central, *S. subniger* South, and *S. subniger* East, and the closely related species *S. bicanthalis* ("outgroup"). Locality data for each sample can be found in Table 2.

(vs. a maximum SVL of 56 mm in *S. subniger* "West" and 47 mm in specimens from the Sierra de Mascota). Differences in leg length (tibia length/head length ratio), and numbers of supralabials, scales bordering the interparietal scale, femoral pores, dorsal scales, scales around midbody, and ventral scales also differentiated the three groups of *S. subniger*. Differences in these and other mensural and meristic characters historically used to distinguish species in the *S. scalaris* group are summarized in Tables 3–4.

Recognition of new species. Genomic and mitochondrial data suggest that gene flow between *S. subniger* "West" and *S. subniger* across the central TVB is limited, as evidenced by a lack of admixture and haplotype sharing between the two lineages. Additionally, mtDNA haplotypes from the Sierra de Mascota in western Jalisco are distantly related to those from other *S. subniger*. Morphological differences are more subtle, although some combinations of morphological characters suggest *S. subniger* "West" and specimens from the Sierra de Mascota are phenotypically divergent from each other and *S. subniger* (Table 3). As noted in previous studies, morphological differences among many species in the *S. scalaris* group are difficult to discern based on traditional characters (Thomas & Dixon 1976; Grummer & Bryson 2014), despite strong genetic differentiation (Bryson *et al*. 2012; Grummer *et al*. 2014; Leaché *et al*. 2016). In these cases, whether genetic differences occur across well-known vicariant barriers can provide another line of evidence that the differences are not clinal but instead differentiate discrete units of biodiversity (e.g., Jadin *et al*. 2012; Reilly *et al*. 2015). *Sceloporus subniger*, *S. subniger* "West", and the population in the Sierra de Mascota each occupy allopatric regions of the TVB (Fig. 1). In the west, large swaths of low-elevation habitat appear to isolate the population in the Sierra de Mascota from the nearest populations of *S. subniger* "West". To the east, a series of drainages that cut into the narrow central part of the TVB appears to separate *S. subniger* "West" from *S. subniger* to the east, a conclusion supported by our genetic data. Co-distributed montane rattlesnakes in the *Crotalus triseriatus* group show a similar geographic pattern, with closely related species split on either side of these geographic breaks (*C. campbelli* and *C. armstrongi* in the west, and *C. armstrongi* and *C. tlaloci* to the east; Bryson *et al*. 2014). Collectively, these lines of evidence from genetics, morphology, and geography support the hypothesis that *S. subniger* "West" and the population from the Sierra de Mascota represent distinct evolutionary lineages with independent evolutionary trajectories, which can now be described as new species.

Sceloporus dixoni **sp. nov. Bryson & Grummer**

Figs. 4–5, Tables 3–4

Sceloporus aeneus – Duellman 1965 (in part)
Sceloporus aeneus – Thomas & Dixon 1976 (in part)
Sceloporus aeneus – Benabib *et al*. 1997 (in part)
Sceloporus aeneus aeneus – Smith 1937 (in part)
Sceloporus aeneus aeneus – Smith 1939 (in part)
Sceloporus aeneus aeneus – Schmidt & Shannon 1947
Sceloporus aeneus aeneus – Duellman 1961 (in part)
Sceloporus aeneus aeneus – Mink *et al*. 1996 (in part)
Sceloporus aeneus subniger – Smith *et al*. 1993 (in part)
Sceloporus aeneus subniger – Bryson *et al*. 2012 (in part)
Sceloporus subniger – Grummer *et al*. 2014 (in part)

Holotype: Adult male, UTA 61714 (field number RWB 0649), from Nevado de Colima, 13.5 mi W Cd. Guzmán, municipality of San Gabriel, Jalisco (N 19.6427°, W 103.6236°, 2375 m; WGS84); collected 24 June 2006 by R. W. Bryson Jr.

Paratypes: same data as holotype (MZFC 22053, 22054; UTA 61713, 61715–61716). Michoacán: 11.7 mi W Zacapu on rd to Zamora (MZFC 22055, 22056; UTA 61699–61702). 22 km N Uruapan on Hwy 37 (UTA 61703, 61704).

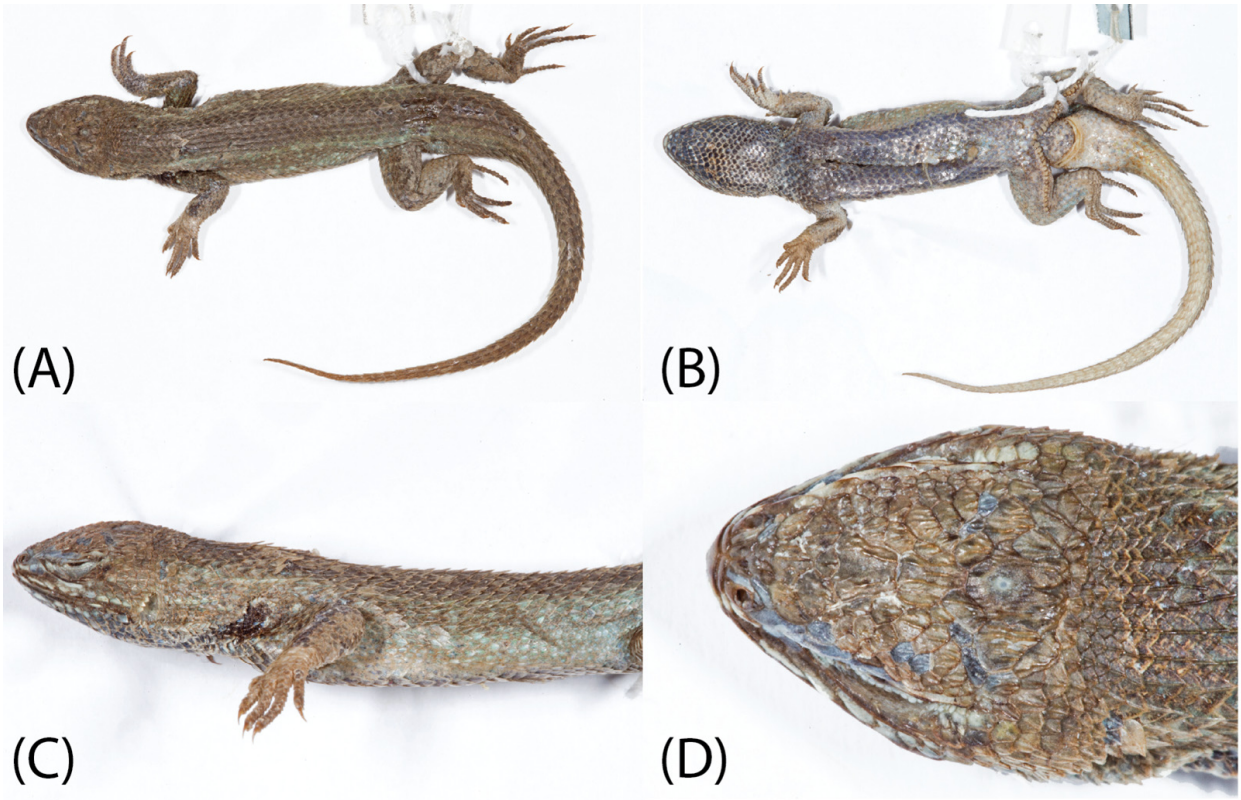

FIGURE 4. (A) Dorsal, (B) ventral, and (C) lateral views of the holotype of *Sceloporus dixoni* sp. nov. (UTA 61714) (D) Close-up view of head.

FIGURE 5. Dorsal and ventral aspects of male *Sceloporus dixoni* sp. nov. from the type locality. Specimen photographed and released.

Diagnosis. *Sceloporus dixoni* is a member of the *S. scalaris* group, sharing with other species in this group parallel lateral scale rows, femoral pore series that are either in contact or separated by no more than two scales, females with smooth preanal scales, and males with lateral abdominal color patches (Smith 1939; Smith *et al*. 1997; Watkins-Cowell *et al*. 2006). *Sceloporus dixoni* can be distinguished from other species in this group by the following combination of characters: single canthal on each side of the head, small adult size (maximum SVL = 54 mm, average 47.1 mm), 37–45 dorsal scales (average 41), 37–43 scales around midbody (average 40), 32–39 ventral scales (average 35), tibia length/head length proportion of 0.76–0.94 (average 0.86), 4–5 supralabial scales (mode of 5), 12–18 scales bordering the interparietal scale (average 15), 31–36 femoral pores in males (average 34), adult females with lightly mottled venters, and adult males with extensive dark pigment on the venter, heavily mottled throats, and orange or rust-colored flanks.

Description of holotype. Adult male (Fig. 4). SVL = 53 mm, total length including tail = 124 mm. Head length = 10.16 mm. Tibia length = 9 mm. Entire hind limb length (including fourth toe) = 21 mm. Forelimb length = 10.6 mm. Dorsal head scales keeled with smooth margins. Four internasal scales about twice as high as wide. Canthals 1-1. Loreals 1-1. Supralabials 5-5. Infralabials 6-6. Postnasals 3-2. Preoculars 1-1, with strong transverse keel on dorsal portion. Three frontonasals, each with >3 ridges. Three prefrontals, two large lateral ones (each with three ridges) and one small medial one with a single ridge. Frontal trapezoidal, with a medial depression and ridges on lateral portions. Frontoparietals 1-1. Parietals 2-1. Lorilabial rows 2-2. Dorsal scales triangular, keeled; about 75% of them possessing a spiny distal projection. Dorsal scale margins smooth (not serrate), transparent. Forty dorsal scales. Forty scales around midbody. Ventral scales rounded with a notch at posterior apex.

Color in preservative. Dorsal and lateral surface of head medium brown. Suboculars, loreals, canthals, and labiomentals white. Throat dark blue/black with about 10 light-colored scales scattered across gular region. Dorsum medium brown, patternless. Lateral areas of body light brown and turquoise. Venter dark with turquoise in posteromedial portion, slightly less melanized near intersection with hind limbs. Dorsal surface of tail medium brown, patternless, turning to light brown towards tail tip; ventral surface cream. Forelimbs same ground color as dorsum; elbows and forearms with turquoise scales. Hindlimbs same color as dorsum.

TABLE 3. Variation in morphological characters examined in male specimens of the montane bunchgrass lizard *Sceloporus subniger* for this study. Range with means in parentheses; italicized characters show range with mode.

Character	***S. subniger* (n=7)**	***S. subniger* "West" (n=6)**	***S. subniger* Sierra de Mascota (n=3)**
Snout–Vent Length	41–60 (49.9)	42–54 (48.2)	43–47 (45)
Head Length	9.2–12.5 (10.9)	9.5–11.5 (10.3)	9.3–10.1 (9.7)
Head Width	8.3–10.8 (9.8)	8.4–10 (9.6)	8.8–9.2 (9)
Head Width / Head Length Ratio	0.85–0.98 (0.9)	0.86–0.98 (0.93)	0.91–0.94 (0.93)
Tibia Length	7–11 (8.6)	8–9 (8.8)	8–9 (8.3)
Tibia Length / Head Length Ratio	0.7–0.88 (0.79)	0.78–0.9 (0.86)	0.83–0.89 (0.86)
Dorsal Scales	39–44 (41)	40–45 (42)	42–47 (45)
Midbody Scales	34–44 (41)	39–43 (40)	40–44 (42)
Ventral Scales	27–42 (34)	32–37 (35)	38–39 (38)
Canthals	1–1 (1)	1–1 (1)	1–1 (1)
Loreals	1–2 (1)	1–1 (1)	1–2 (1)
Supralabials	4–5 (4)	4–5 (5)	4–5 (4)
Infralabials	5–6 (6)	6–7 (6)	5–7 (6)
Frontoparietals	1–2 (1)	1–2 (1)	1–1 (1)
Parietals	1–1 (1)	1–1 (1)	1–1 (1)
Scales Bordering Interparietal	14–17 (16)	13–18 (15)	11–17 (14)
2nd Pair of Postmentals	1–2 (2)	0–2 (2)	1–2 (2)
3rd Pair of Postmentals	3–5 (5)	2–5 (5)	4–4 (4)
Femoral Pores	32–40 (35)	31–36 (34)	34–36 (35)
Fourth Toe Lamellae	15–21 (17)	16–22 (19)	16–20 (17)

Variation. Variation in meristic and mensural characters of male and female paratypes is summarized in Tables 3–4. All males have heavily mottled throats; in several, the mottling is so dense that the ventral surface of the head appears almost entirely black, as seen in the holotype. Ventral surfaces of males are similarly dark in preservative; in some, a pale-colored patch extends midventrally from about the intersection of the hindlimbs towards the front limbs. This lighter-colored section of the venter is especially evident in life, as seen in Fig. 5. Also noticeable in this

image are the lateral blue patches on the venter and orange-red color of the flanks of males. In preservative, the ventral surface darkens considerably, presumably due to fixation in formalin. The dorsal surface of males ranges from weakly patterned to patternless. When patterned, the dorsal surface is marked by a pair of light-colored dorsolateral stripes, one-scale wide, that originates at the posterior margin of ear opening and extends onto the tail. A pale vertebral line, two scale-rows wide, is also present, beginning at the nape of the neck and extending posteriorly to tail. The region between the vertebral and dorsolateral stripe is marked with narrow, dark brown transverse bars on each side; in many individuals, these bars are dimly evident. Females possess lightly mottled throats, some with more mottling than others. The ventral surface of females is very lightly mottled. The dorsal surface of females ranges from strongly patterned to patternless. In strongly patterned individuals, dark transverse bars are sharply defined, often edged posteriorly by white.

TABLE 4. Variation in morphological characters examined in female specimens of the montane bunchgrass lizard *Sceloporus subniger* for this study. Range with means in parentheses; italicized characters show range with mode.

Character	***S. subniger* (n=21)**	***S. subniger* "West" (n=8)**	***S. subniger* Sierra de Mascota (n=2)**
Snout–Vent Length	41–62 (48.1)	42–52 (46.3)	45–47 (46)
Head Length	8.7–11.8 (9.8)	8.7–10.2 (9.5)	9.2–9.4 (9.3)
Head Width	7.8–10.7 (8.9)	8–9.7 (8.7)	8.4–8.8 (8.6)
Head Width / Head Length Ratio	0.85–1.04 (0.91)	0.84–0.98 (0.92)	0.9–0.96 (0.93)
Tibia Length	7–10 (8.3)	7–9 (8.3)	7–8 (7.5)
Tibia Length / Head Length Ratio	0.76–0.92 (0.85)	0.76–0.94 (0.87)	0.75–0.87 (0.81)
Dorsal Scales	37–46 (40)	37–42 (40)	41–42 (41)
Midbody Scales	37–44 (41)	37–42 (39)	43–45 (44)
Ventral Scales	31–41 (34)	32–39 (35)	37–39 (38)
Canthals	1–1 (1)	1–1 (1)	1–1 (1)
Loreals	1–2 (1)	1–2 (1)	1–1 (1)
Supralabials	3–5 (4)	4–5 (4)	4–5 (4)
Infralabials	5–6 (6)	4–6 (6)	5–6 (6)
Frontoparietals	1–2 (1)	1–2 (1)	1–1 (1)
Parietals	1–2 (1)	1–1 (1)	1–1 (1)
Scales Bordering Interparietal	14–17 (15)	12–17 (14)	15–15 (15)
2nd Pair of Postmentals	0–3 (2)	1–2 (2)	1–2 (1, 2)
3rd Pair of Postmentals	3–6 (5)	4–5 (4)	3–4 (3, 4)
Fourth Toe Lamellae	15–22 (18)	16–21 (19)	18–18 (18)

Comparisons. *Sceloporus dixoni* is most similar to *S. subniger* and specimens from the Sierra de Mascota in western Jalisco, sharing with them a single canthal on each side of the head, relatively short legs (average tibia length/head length proportion less than 0.9), small adult size (maximum SVL less than 63 mm), 36–50 dorsal scales, extensive dark pigment on the venter of adult males, a black-barred or darkly mottled chin/throat in adult males, orange or rust-colored flanks in adult males, and oviparity. *Sceloporus dixoni* can be distinguished from *S. subniger* by the combination of its smaller adult size (maximum SVL = 54 mm in *S. dixoni* vs. 62 mm in *S. subniger*; average SVL = 47.1 mm vs. 48.6 mm), longer legs (average tibia length/head length proportion 0.86 vs. 0.83), fewer femoral pores in males (maximum of 36 vs. 40; average number 34 vs. 35), fewer scales around midbody (average of 40 vs. 41), more supralabial scales (mode of 5 vs. 4), and fewer scales bordering the interpariatel scale (average of 15 vs. 16). Female *S. dixoni* also have considerably less mottling on the ventral surface than female *S. subniger*. *Sceloporus dixoni* differs from specimens from the Sierra de Mascota in western Jalisco by the combination of their larger adult size (maximum SVL = 54 mm in *S. dixoni* vs. 47 mm in specimens from the Sierra de Mascota; average

SVL = 47.1 mm vs. 45.4 mm), slightly longer legs (average tibia length/head length proportion 0.86 vs. 0.84), fewer ventral scales (a minimum of 32 vs. 37; average = 35 vs. 38), fewer dorsal scales (37–45, average = 41 vs. 41–47, average = 43), and fewer scales around midbody (37–43, average = 40 vs. 40–45, average = 43).

Etymology. The specific epithet is a patronym honoring the late James R. Dixon for his decades of research on Mexican herpetofauna, including several insightful studies of the *S. scalaris* group. "Doc" Dixon took an early interest in the academic growth of the first author and made a profound and lasting impact. For this and for his encouragement and support, he will be truly missed.

Distribution. *Sceloporus dixoni* is distributed in primarily pine-oak forest along the western half of the Trans-Mexican Volcanic Belt, from near Morelia, Michoacán, to the lower slopes of the Nevado de Colima in Jalisco. East of Morelia, the series of steep low-elevation drainages leading into the Balsas Basin likely serve as a geographic barrier between *S. dixoni* to the west and *S. subniger* to the east (Fig. 1).

Comments. Several species in the *S. scalaris* group form a distinct subgroup based on morphology (Smith *et al*. 1993) and genetic data (Mink & Sites 1996; Benabib *et al*. 1997; Bryson *et al*. 2012; Grummer *et al*. 2014; Leaché *et al*. 2016), including *S. aeneus* Wiegmann, *S. bicanthalis*, *S. subniger*, *S. dixoni*, and specimens from the Sierra de Mascota in western Jalisco. *Sceloporus bicanthalis* is the only species in this subgroup that is viviparous and that has two vs. one canthal scales on each side of the head. Confusion regarding parity in these species was clarified by Méndez-de la Cruz *et al*. (1998). All species inhabit montane bunchgrass meadows along the length of the Trans-Mexican Volcanic Belt of Mexico.

The taxonomic placement of *S. subniger* has varied since its description as a subspecies of *S. aeneus* (Poglayen & Smith 1958). Thomas & Dixon (1976) argued that *S. a. aeneus* and *S. s. subniger* were indistinguishable. Smith *et al*. (1993) challenged this conclusion, claiming it was based on misidentified specimens from Nevado de Toluca and therefore an inaccurate description of the status and distribution of *S. a. subniger*. *Sceloporus subniger* and *S. aeneus* were subsequently considered distinct species in checklists (Liner 1994; Bell *et al*. 2003), a taxonomic proposal consistent with multilocus genetic data (Grummer *et al*. 2014). Based on molecular data (Bryson *et al*. 2012; Grummer *et al*. 2014), the distribution of *S. aeneus* is certainly much smaller than envisioned by Smith in his early studies (e.g., Poglayen & Smith 1958). This smaller distribution is more accurately reflected in Smith's later maps (e.g., Smith *et al*. 1993). The absence of a black-barred or mottled chin/throat and smaller adult size may distinguish *S. aeneus* from *S. subniger* (Smith *et al*. 1993).

Sceloporus hesperus sp. nov. Bryson & Grummer

Figs. 6–7, Tables 3–4

Holotype: Adult male, MZFC 35571 (field number RWB 1107), from 2.2 km (by air) SE Lago de Juanacatlán, Sierra de Mascota, municipality of Mascota, Jalisco (N 20.6102°, W 104.7208°, 2314 m; WGS84); collected 10 April 2011 by R. W. Bryson Jr. and M. Torocco.

Paratypes: Same data as holotype (MZFC 35572–35575).

Diagnosis. *Sceloporus hesperus* is a member of the *S. scalaris* group, sharing with other species in that group parallel lateral scale rows, femoral pore series that are either in contact or separated by no more than two scales, females with smooth preanal scales, and males with lateral abdominal color patches (Smith 1939; Smith *et al*. 1997; Watkins-Cowell *et al*. 2006). *Sceloporus hesperus* can be distinguished from other species in this group by the following combination of characters: single canthal on each side of the head, small adult size (SVL less than 47 mm, average 45.4 mm), 41–47 dorsal scales (average 43), 40–45 scales around midbody (average 43), 37–39 ventral scales (average 38), tibia length/head length proportion of 0.75–0.89 (average 0.84), 11–17 scales bordering the interpariatel scale (average 14), adult females with lightly mottled or pale venters, and adult males with extensive dark pigment on the venter, heavily mottled throats, and orange or rust-colored flanks.

Description of holotype. Adult male (Fig. 6). SVL = 43 mm, total length including tail = 112 mm. Head length = 9.64 mm. Tibia length = 8 mm. Entire hind limb length (including fourth toe) = 21 mm. Forelimb length = 11 mm. Dorsal head scales keeled with smooth margins. Four internasal scales, two deeply notched towards midline and two rectangular. Canthals 1-1. Loreals 1-1. Supralabials 5-4. Infralabials 6-6. Postnasals 2-3. Preoculars 1-1 with strong transverse keel on dorsal surface. Three frontonasals, each with many ridges. Three prefrontals, each with many ridges. Frontal trapezoidal, with three ridges. Frontoparietals 1-1. Parietals 2-1. Lorilabial rows 2-2. Dorsal scales keeled, triangular, with sharp posterior point. Dorsal scale margins transparent, smooth (not serrate). Forty two dorsal scales. Forty scales around midbody. Ventral scales rounded, with a notch at posterior apex.

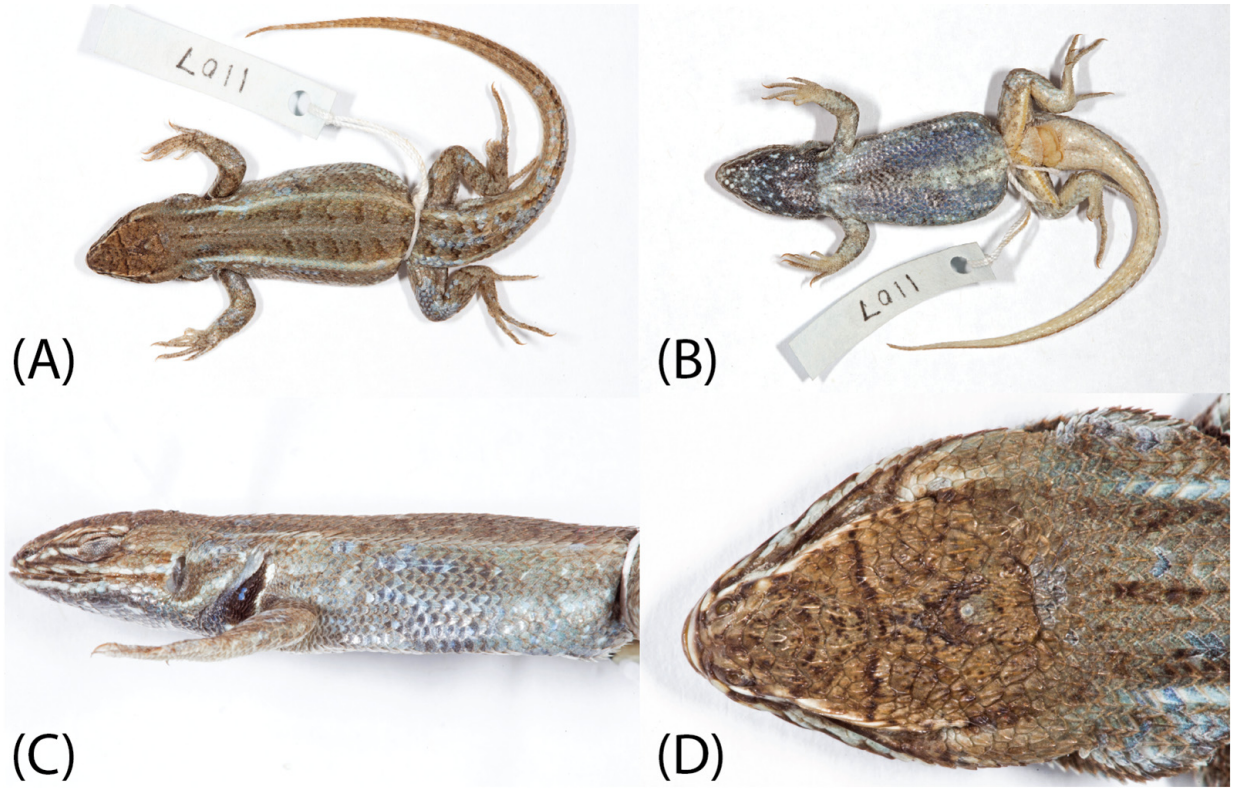

FIGURE 6. (A) Dorsal, (B) ventral, and (C) lateral views of the holotype of *Sceloporus hesperus* sp. nov. (MZFC 35571). (D) Close-up view of head.

Two white dorsolateral stripes, one-scale wide, that begin at posterior margin of ear opening and extend posteriorly to tail. Pale brown vertebral stripe two scale-rows wide. Five dark brown transverse bars between vertebral and dorsolateral stripe on each side. Gular region mostly black with about 10% of scales white, randomly scattered. Proximal dorsal surface of tail with four dark brown blotches on either side of midline; few blue scales scattered on proximal half.

Color in preservative. Dorsal and lateral surface of head brown; labials dark brown, bordered dorsally and ventrally by one row of white scales. Loreal scales white. Dorsum light brown, with dark brown transverse bars and light dorsolateral stripes as described above. All white regions on dorsum infused with blue (presumably from leeching). Lateral body scales light, with increasing melanism towards venter. Throat dark blue or black, with few, scattered light-colored scales. Venter dark blue and melanized, except for midventral, pale-colored patch about six scale-rows wide extending anteriorly from inguinal region, diminishing anteriorly until absent on chest. Dorsal surface of tail as above; ventral surface cream. Limbs same color as dorsum, with few scattered light blue scales.

Variation. Variation in meristic and mensural characters of male and female paratypes is summarized in Tables 3–4. All males have heavily mottled, dark turquoise throats. Ventral surfaces of two males (including the holotype) are similarly dark in preservative, with a distinct pale-colored patch extending midventrally from about the intersection of the hindlimbs towards the front limbs; in the third, the belly is much less melanized. The dorsal surface of males ranges from weakly patterned to patternless. In weakly patterned individuals, such as the holotype, the dark brown transverse bars between the vertebral and dorsolateral stripes are dimly evident. Figure 7 shows the coloration of an adult male in life; particularly noticeable are the orange-colored flanks of males. Of the two paratype females, one has a lightly mottled throat and ventral surface, while the other is pale. The dorsal surface of one female is strongly patterned, and marked with sharply defined dark transverse bars; the other female is patternless.

FIGURE 7. Male *Sceloporus hesperus* sp. nov. from the type locality. Specimen photographed and released.

Comparisons. *Sceloporus hesperus* is one of the smallest species in the *S. scalaris* group, having a mean SVL of 45.4 mm. *Sceloporus chaneyi*, previously reported to be the smallest *S. scalaris* group species, has a mean SVL of 45.7 mm (Liner & Dixon 1992). *Sceloporus hesperus* is most similar to *S. subniger* and *S. dixoni*, sharing with them a single canthal on each side of the head, relatively short legs (tibia length/head length proportion less than 0.9), small adult size (maximum SVL less than 63 mm), 36–50 dorsal scales, extensive dark pigment on the venter of adult males, a black-barred or darkly mottled chin/throat in adult males, orange or rust-colored flanks in adult males, and oviparity. *Sceloporus hesperus* can be distinguished from *S. subniger* by the combination of its smaller adult size (maximum SVL = 47 mm in *S. hesperus* vs. 62 mm in *S. subniger*; average SVL = 45.4 mm vs. 48.6 mm), more dorsal scales (average of 43 vs. 41), more scales around midbody (average of 43 vs. 41), more ventral scales (average of 38 vs. 34), and fewer scales bordering the interpariatel scale (average of 14 vs. 16). Female *S. hesperus* also have considerably less mottling on the ventral surface than female *S. subniger*. *Sceloporus hesperus* differs from *S. dixoni* by the combination of their smaller adult size (maximum SVL = 47 mm in *S. hesperus* vs. 54 mm in *S. dixoni*; average SVL = 45.4 mm vs. 47.1 mm), slightly shorter legs (average tibia length/head length proportion 0.84 vs. 0.86), more ventral scales (average = 38 vs. 35), more dorsal scales (41–47, average = 43 vs. 37–45, average = 41), and more scales around midbody (40–45, average = 43 vs. 37–43, average = 40).

Etymology. The specific epithet is derived from the Greek word *hesperos,* meaning "western", and is used in reference to the type locality located at the far western end of the Trans-Mexican Volcanic Belt.

Distribution. Smith (1939: 356) cites several published records from the late 1800s for *S. aeneus aeneus* from Jalisco: "N of Rio Santiago (Günther 1890); La Cumbre de los Arrastrados (Boulenger 1897); Hda. Santa Gertrudis (Boulenger 1897)." Over 50 years later, Smith *et al*. (1993: 133) commented that Boulenger's records from Jalisco "appear to be too far west to be accepted without verification." If Boulenger's records are indeed correct, specimens from La Cumbre de los Arrastrados and Hacienda Santa Gertrudis in the Sierra de Cuale might be referable to *S. hesperus*. A narrow ridge above 2,200 m extends across southwestern Jalisco from the type locality in the Sierra de Mascota through the Sierra de Cuale to the Sierra de Manantlán. The reptiles and amphibians in this remote region of Mexico remain poorly sampled (Reyes-Velasco *et al*. 2010). If suitable montane bunchgrass habitat is present along the forested mountain pass connecting these three sierras, then it is conceivable that additional populations of *S. hesperus* will be found here. For now, the only known population of *S. hesperus* is from the high-elevation pine-oak forest at the type locality in the Sierra de Mascota of Jalisco. The low-elevation valleys trending northwest from

the Nevado de Colima likely serve as a geographic barrier between *S. hesperus* and *S. dixoni*. This lower-elevation area is also inhabited by *S. unicanthalis* and *S. scalaris* (Thomas & Dixon 1976; Watkins-Colwell *et al*. 2006), two larger-bodied species in the *S. scalaris* group not known to co-occur with *S. subniger*.

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APPENDIX 1. Specimens examined for morphological comparisons.

Sceloporus dixoni. **Jalisco:** Nevado de Colima, 13.5 mi W Cd. Guzmán, municipality of San Gabriel, Jalisco (MZFC 22053, 22054; UTA 61713, 61715–61716). **Michoacán**: 11.7 mi W Zacapu on rd to Zamora (MZFC 22055, 22056; UTA 61699–61702). 22 km N Uruapan on Hwy 37 (UTA 61703, 61704).

Sceloporus hesperus. **Jalisco:** 2.2 km (by air) SE Lago de Juanacatlán, Sierra de Mascota, municipality of Mascota (MZFC 35571–35575).

Sceloporus subniger. **Estado de México**: 3.2 mi SW Atlacomulco (MZFC 22059, 22060; UTA 61695–61698). Los Tachos (UTA 61691–61694). Raices, 4.4 km (by road) N of, along Mexico Highway 3 (USNM 204972–204974). Zitácuaro, 18 mi E of (USNM 148557, 148558). Joquicingo (MZFC 35576, 35577). **Michoacán**: 8 mi NW Ciudad Hidalgo (MVZ 71940–71947). 7 mi NW Zitácuaro (TCWC 35225, 35226, 36858).